

Polymorphism of Lampbrush Chromosomes in Japanese Populations of *Rana nigromaculata*

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ABSTRACT—The 13 pairs of the lampbrush chromosomes of *R. nigromaculata* are characterized by one to five landmarks situated at specific positions on the axis of each chromosome. In *R. nigromaculata* collected from 28 sites in Japan, eight chromosome pairs did not show any variations, whereas the remaining five pairs consisted of two forms of chromosome which differed in the number or type of the landmarks. The variations in chromosomal polymorphism indicated that Japanese *R. nigromaculata* has genetically differentiated into four groups which were formed by a break in the migration due perhaps to geographic obstacles and the expansion of new genetic materials provided from continental *R. nigromaculata* in the Würm glacial stage. By comparing the distribution of the variations and the lampbrush chromosomes of continental *R. nigromaculata*, the history of the migration of *R. nigromaculata* into Japan is discussed.

INTRODUCTION

Rana nigromaculata is distributed over the north-eastern area of China, the whole of the Korean Peninsula, and Kyushu, Shikoku and Honshu (except the Kanto and Sendai plains) in Japan [5]. This species is supposed to have evolved from an ancestral species population common to *Rana plancyi* in the continent, to adapt to the arid and cold climates [6]. After its own evolution was sufficiently completed, *R. nigromaculata* came to Japan through the Korean Peninsula when Japan was still a part of the continent [6]. Recently, Nishioka *et al.* [12] found that *R. nigromaculata* collected from 45 sites in Japan have differentiated into four groups on the basis of Nei's genetic distances obtained from electrophoretic analyses.

The genetic differentiation was reflected in the characteristics of its lampbrush chromosomes which are composed of 13 pairs of bivalent chromosomes at the diplotene stage of oogenetic meiosis. The lampbrush chromosomes of *R. nigromaculata* are characterized by one to five landmarks, consisting of simple-type giant loops, compound-type giant loops, spheres, and an oval-like structure, at specific positions of each chromosome axis [10], though the landmarks grow conspicuous by accumulating their own gene product around their axes [1]. However, some lampbrush chromosomes showed variations in the landmarks among populations of *R. nigromaculata*. These variations are presumed to relate to the processes of the habitat expansion of *R. nigromaculata* in Japan in the same way as the mitotic chromosomal mutations of *Rattus* species [15-17].

Herein, the geographical distribution and the history of the polymorphism of the lampbrush chromosomes of Japanese *R. nigromaculata* in comparison with those of continental species are described.

MATERIALS AND METHODS

Lampbrush chromosomes were removed from the ova of 199 female *Rana nigromaculata* Hallowell collected from 28 sites in Japan. Figure 1 shows the collection sites and the number of females. In addition, lampbrush chromosomes were removed from three females from Beijing, China, and six females from Suwon, Korea, which had been bred in the Laboratory for Amphibian Biology, Hiroshima University.

Lampbrush chromosomes were prepared by means of a slight modification of Gall's method [2, 13]. Because the size of the landmarks varies with the stages of oogenesis, ten or more lampbrush chromosome preparations per female were examined under a phase-contrast microscope to avoid misjudgements on the presence or absence of landmarks.

RESULTS

Previously, Nishioka *et al.* [10] described a map of the lampbrush chromosomes of *R. nigromaculata*, which represented the positions and types of landmarks on each chromosomal axis. The map was constructed from the lampbrush chromosomes of three female offspring of a pair collected from Hiroshima. According to that map, 13 lampbrush chromosomes possess one to five landmarks each, or a total of 35 landmarks, and they are all distinguishable by position and type.

In this study, eight of the 13 lampbrush chromosomes of all the populations of Japanese *R. nigromaculata* examined showed the same characteristics (2-5, 8-10, and 12). By contrast, the remaining five, 1, 6, 7, 11, and 13, showed two forms which differed in the number or type of the landmarks they carried. In chromosome 1, one form possessed two simple-type giant loops, one on the short arm and the other on the long arm; the other form possessed one compound-type giant loop on the long arm in addition to these (Fig. 2a). In chromosome 6, one form had one compound-type and one simple-type giant loop on the long arm; the other had another

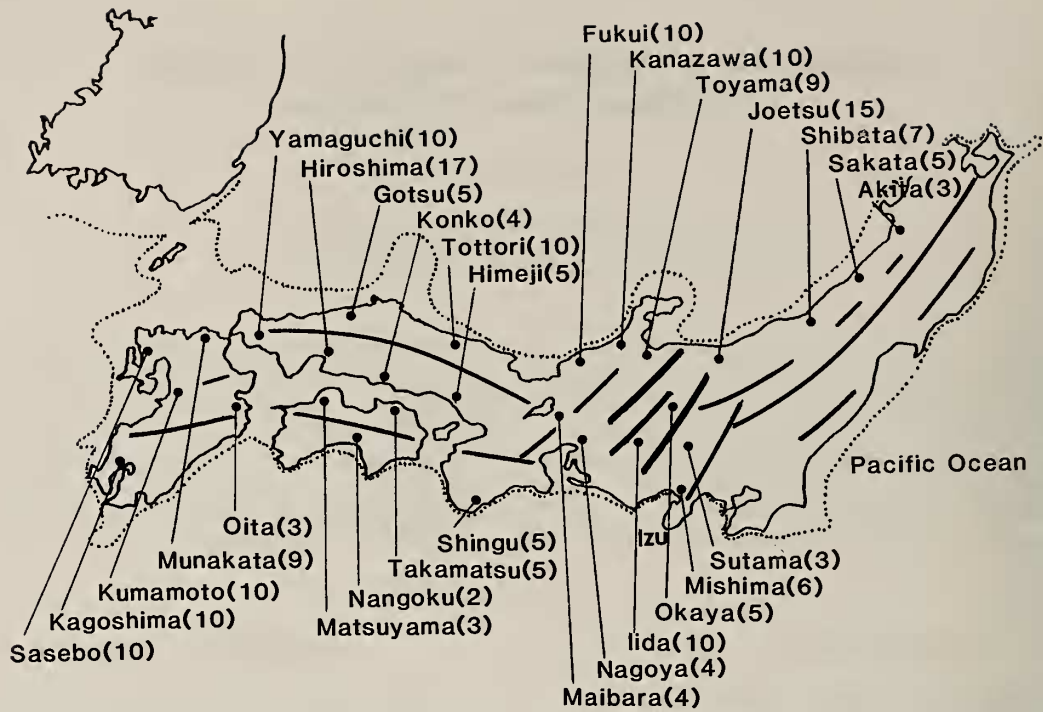


FIG. 1. Map showing the collection sites of *Rana nigromaculata* and the number of females studied. Bold lines mark the mountain ranges and a dotted line marks the location of the presumptive coastline about 2×10^4 years ago [3].

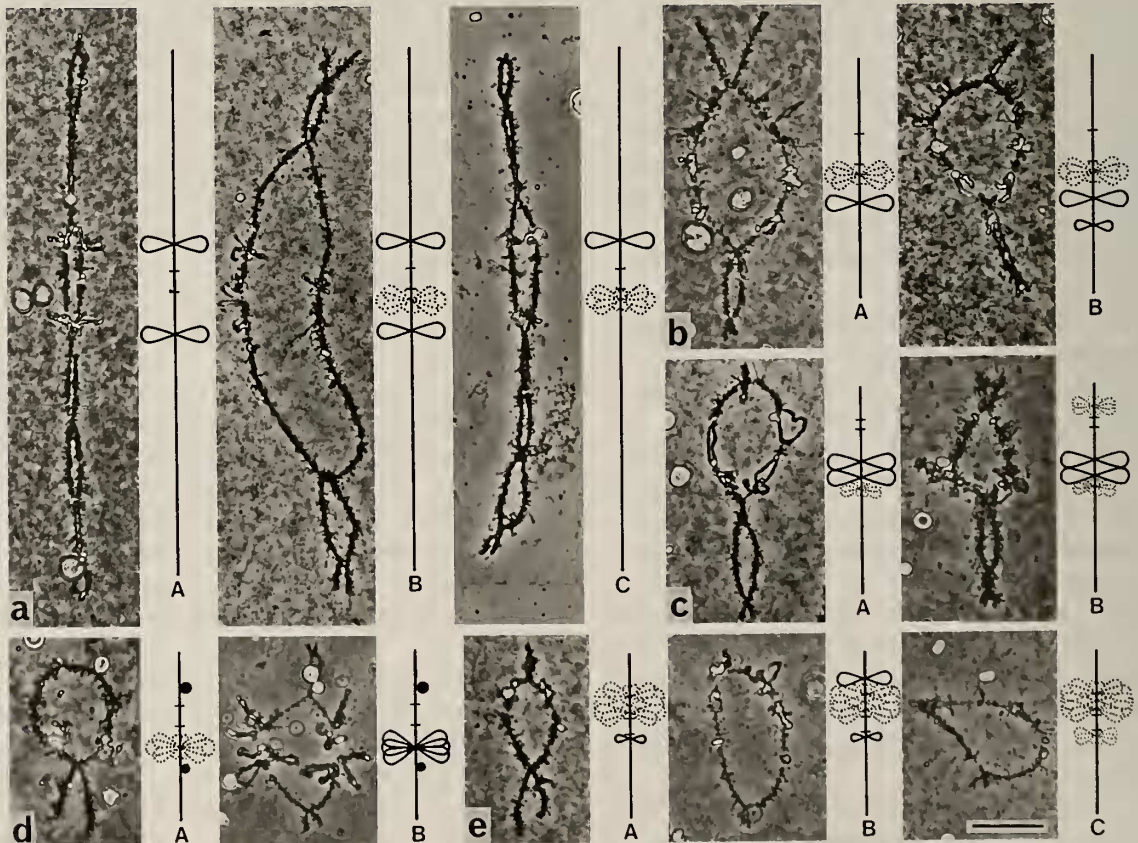


FIG. 2. Variations in lampbrush chromosomes 1(a), 6(b), 7(c), 11(d), and 13(e) of *R. nigromaculata*. In diagrammatic representation, simple- and compound-type giant loops are represented by solid and dotted lines, respectively, and spheres are shown with black circles. A segment between two short parallel lines shows a range consisting of larger normal loops than any other parts and including a centromere. A, B, and C indicate A-, B-, and C-form, respectively. In photographs, simple-type giant loops appear as a stiff loop which becomes thick by covering its axis with a large quantity of matrixes and it has a smooth outline. Compound-type giant loops consist of two or more simple-type giant loops which are more slender than the independent simple-type giant loops and have a notched outline. Scale bar represents $100 \mu\text{m}$.

simple-type giant loop on the long arm in addition to these (Fig. 2b). In chromosome 7, one form had two simple-type and one compound-type giant loop on the long arm; the other had another compound-type giant loop on the short arm in addition to these (Fig. 2c). In chromosome 11, one form had one compound-type giant loop on the long arm and two spheres on both the arms; the other had three simple-type giant loops in place of the compound-type giant loop of the former (Fig. 2d). In chromosome 13, one form had one compound-type giant loop on the short arm and one simple-type giant loop on the long arm; the other had another simple-type giant loop on the short arm in addition to these (Fig. 2e). In each of these five chromosomes, the former was named the A-form, and the latter was the B-form.

Based upon the frequencies of the A- and B-forms in each of the 28 collection sites, the *R. nigromaculata* populations were divided into four groups, named the Eastern Honshu, Chubu, Western Honshu-Shikoku, and Kyushu groups, respectively (Fig. 3; Table 1). In chromosome 1, the A-form was predominant in the Chubu group, while the B-form was predominant in the Eastern Honshu and Western Honshu-Shikoku groups. In the Kyushu group, the frequency of the B-form was a little higher than that of the A-form. It was remarkable, however, that nine of the 10 females from Kagoshima had the homologous A-form.

In chromosome 6, the two forms were somewhat similar in distributional pattern to those of chromosome 1. The A-form was found in the Chubu group at a fairly high frequency, not being so predominant as the A-form of chromosome 1. The B-form was predominant in the Eastern Honshu and Western Honshu-Shikoku groups, like chromosome 1. The Kyushu group also involved the B-form at high frequency. It was remarkable, however, that five females from Kagoshima were homozygous for the A-form and four were heterozygous for the A- and B-forms.

In chromosome 7, all the females of the Eastern Honshu, Chubu, and Western Honshu-Shikoku groups had the only A-form. On the other hand, the Kyushu group had the B-form at a nearly equal frequency to that of the A-form. It was remarkable, however, that all females from Kagoshima had the homologous A-form.

In chromosome 11, the B-form was distributed in a similar manner to the A-form of chromosome 7. The B-form was found in all the females of the Eastern Honshu, Chubu, and Western Honshu-Shikoku groups in the homologous condition. The Kyushu group involved both the A- and B-forms at nearly equal frequencies.

In chromosome 13, all the females of the Eastern Honshu and Chubu groups were homologous for the A-form. On the other hand, most of the females of the Western

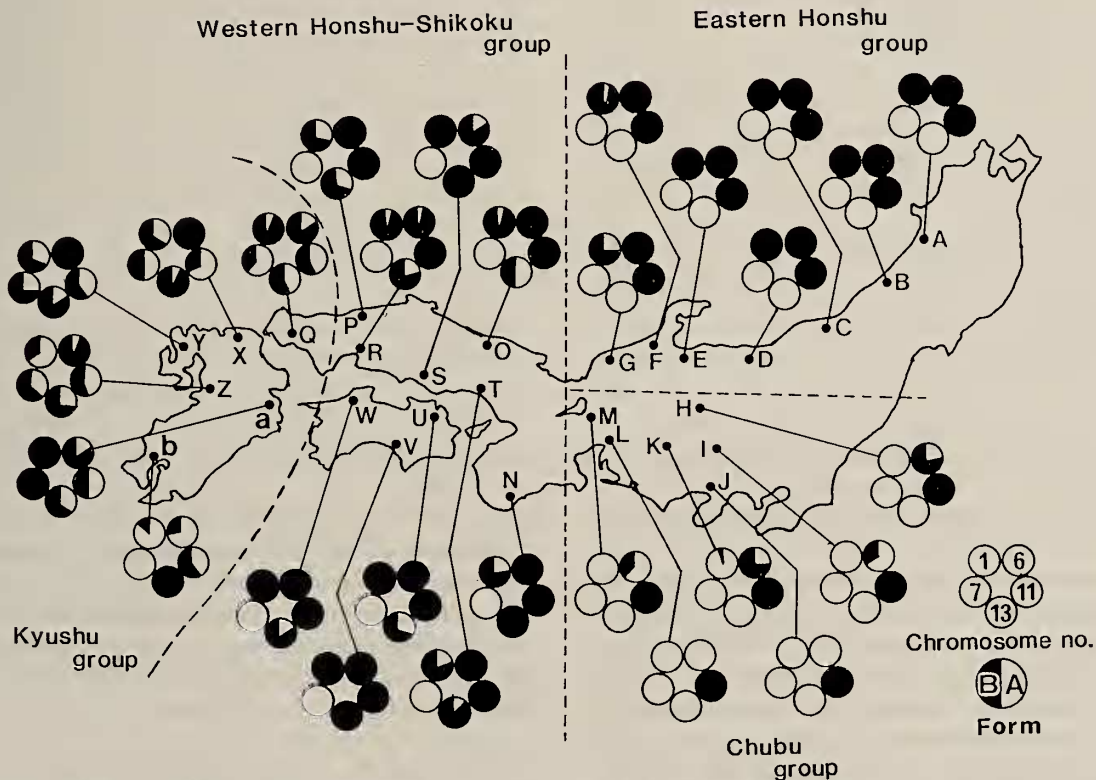


FIG. 3. Frequencies of the A- and B-form of five lampbrush chromosomes, 1, 6, 7, 11, and 13, in each collecting site. Areas partitioned with broken lines represent groups of *R. nigromaculata* females with about the same characteristics in their lampbrush chromosomes. A, Akita. B, Sakata. C, Shibata. D, Joetsu. E, Toyama. F, Kanazawa. G, Fukui. H, Okaya. I, Sutama. J, Mishima. K, Iida. L, Nagoya. M, Maibara. N, Shingu. O, Tottori. P, Gotsu. Q, Yamaguchi. R, Hiroshima. S, Konko. T, Himeji. U, Takamatsu. V, Nangoku. W, Matsuyama. X, Munakata. Y, Sasebo. Z, Kumamoto. a, Oita. b, Kagoshima.

TABLE 1. Frequencies of bivalent chromosomal types in four groups of *R. nigromaculata*. Figures in parentheses show expected values based on Hardy-Weinberg hypothesis.

Type	Eastern Honshu	Chubu	Western Honshu Shikoku	Kyushu	Total
Chromosome 1					
AA	0 (0.2)	31 (31.0)	1 (0.4)	19 (9.7)	51
AB	6 (5.7)	1 (1.0)	8 (9.1)	7 (25.5)	22
BB	53 (53.2)	0 (0.0)	47 (46.4)	26 (16.7)	126
Total	59	32	56	52	199
Chromosome 6					
AA	0	12 (10.1)	0 (0.0)	5 (1.7)	17
AB	0	12 (15.8)	2 (2.0)	9 (15.5)	23
BB	59	8 (6.1)	54 (54.0)	38 (34.7)	159
Total	59	32	56	52	199
Chromosome 7					
AA	59	32	56	18 (14.0)	165
AB	0	0	0	18 (26.0)	18
BB	0	0	0	16 (12.0)	16
Total	59	32	56	52	199
Chromosome 11					
AA	0	0	0	17 (11.5)	17
AB	0	0	0	15 (25.9)	15
BB	59	32	56	20 (14.5)	167
Total	59	32	56	52	199
Chromosome 13					
AA	59	32	4 (3.0)	4 (1.9)	99
AB	0	0	18 (20.0)	12 (16.2)	30
BB	0	0	34 (33.0)	36 (33.9)	70
Total	59	32	56	52	199

Honshu-Shikoku and Kyushu groups were homozygous for the B-form.

The lampbrush chromosomes of continental *R. nigromaculata* were identical in characteristics with those of Japanese *R. nigromaculata*, except for chromosome 1. The characteristics of chromosomes 6, 7, 11, and 13, in which Japanese *R. nigromaculata* had variations, were of the A-form. However, of the six females from Suwon, two had the homozygous and heterozygous B-form in chromosome 6 and one was homozygous for the B-form in chromosome 11. Chromosome 1 of all the females from Beijing and Suwon had one simple-type giant loop on the short arm and one compound-type giant loop on the long arm (Fig. 2a). This was named the C-form. Of the two landmarks, the former was also

found in the A- and B-forms, and the latter in the B-form. In chromosome 13 of three females from Beijing, there was a variation which was not found in Japanese *R. nigromaculata*. This variation had two compound-type giant loops on the short and long arms and was named the C-form (Fig. 2e). Two females were homologous for the C-form and one was heterologous for the A- and C-forms.

DISCUSSION

Nishioka *et al.* [12] found that *R. nigromaculata* collected from all over Japan can be divided into four groups by a cluster analysis of the genetic distances between local populations. The geographic areas of the four groups are as

follows: 1) the Tohoku area comprising the prefectures of Aomori, Akita, and Niigata; 2) the Chubu area comprising the prefectures of Shizuoka, Aichi, Nagano, and Yamanashi; 3) the Hokuriku, Kinki, Sanyo, Shikoku, and Kagoshima areas comprising the prefectures of Toyama, Ishikawa, Fukui, Shiga, Mie, Wakayama, Osaka, Hyogo, Okayama, Hiroshima, Kagawa, Kochi, Ehime, and Kagoshima; and 4) the San-in and Kyushu areas comprising the prefectures of Tottori, Shimane, Yamaguchi, Fukuoka, Nagasaki, Kumamoto, Oita, and Miyazaki. In this study, it was found that the spread of variations in five lampbrush chromosomes, 1, 6, 7, 11, and 13, is also coincident with this grouping, though there were slight discrepancies in dividing lines. In the four groups based on the chromosomal variations, the geographical area of the Eastern Honshu group comprises the Tohoku and Hokuriku areas, that of the Chubu group comprises the Chubu area, that of the Western Honshu-Shikoku group comprises the Kinki, Sanyo, San-in (except Yamaguchi Prefecture) and Shikoku areas, and that of the Kyushu group comprises the Kyushu area and Yamaguchi and Kagoshima Prefectures.

In each group, the frequencies of the forms of lampbrush chromosomes agreed well with expected values based on the Hardy-Weinberg hypothesis ($P > 0.05$), except for chromosomes 1, 6, and 11 of the Kyushu group. This seems to show that three groups other than the Kyushu group are geographically isolated from one another by major mountain ranges. The Kyushu group, however, is not partitioned from the adjoining group by a clear topographical obstacle, since a part of this group encroaches on the western Honshu area. Moreover, this group seems to include a heterogeneous subgroup, because in chromosomes 1 and 6 many *R. nigromaculata* from Kagoshima had the A-forms which were also found in the Chubu group. The Kyushu group may be divided into two subgroups by increasing the sites of examination further.

It seems that the estimation of the period when the ancestral population of *R. nigromaculata* invaded Japan gives an important clue to the process of the formation of the four groups. Japan was a part of the continent up to the end of the Riss glacial stage [4]. It is reasonably certain that the complete separation of Japan occurred in the Riss-Würm interglacial stage (about 0.12 million years ago) [8]. After entering Japan, *R. nigromaculata* is supposed to have moved along the coastal plains without passing over the highly upheaved mountain ranges; the coastal plains which had widened owing to marine regression in the glacial periods were more favorable. The population which had reached the Chubu area, however, could not go further than this area. The reason is that a collision between the central Honshu region and Izu island (presently a peninsula) broke down the coastal plain (about 0.5 million years ago) [7]. The difficulty of migration in this region is evidenced by the cluster analyses of genetic distances in the populations of *Rana rugosa*, *Rana japonica*, and *Rana brevipoda* [9, 11, 12]. The populations of these species in the Kanto and Sendai plains, which are

situated in the Pacific side of eastern Honshu, are divided in the dendrograms from those in the other areas. Consequently, the absence of *R. nigromaculata* in the Kanto and Sendai plains implies that its ancestral population reached Japan through the Korean Peninsula between about 0.5 and 0.12 million years ago. The area of the north end of the eastern Honshu region could not provide another entrance because the mountain ranges virtually reached the shore.

The formation of the Eastern Honshu, Chubu, and Western Honshu-Shikoku groups is attributable to the release of migration due to marine regression during the glacial stage and the geographical isolation due to marine transgression during the interglacial stages. The formation of the Kyushu group is attributed to the introgression of new genetic materials provided from continental *R. nigromaculata* which invaded again during the Würm glacial stage when the Japanese Islands were temporarily reconnected with the Korean Peninsula (between about 20,000 and 18,000 years ago) [14]. This introgression is typified by the presence of the A-form of chromosome 11 which is found in continental *R. nigromaculata*.

In each of the lampbrush chromosomes 6, 7, 11 and 13, it is quite certain that the A-form is older than the B-form, that is, the B-form was derived from the A-form by mutations. The reasons are, firstly, that continental *R. nigromaculata* is of the A-form in these lampbrush chromosomes and the Chubu group, of which the *R. nigromaculata* migrates to the furthest area in Japan, also has the A-form in the lampbrush chromosomes 6, 7, and 13. Secondly, the lampbrush chromosomes 6, 11 and 13 of *R. brevipoda*, which diverged from an ancestral species common to *R. nigromaculata* [6], have the same characteristics as the A-form [10, 13]. In the two forms of lampbrush chromosome 1, an age comparison is difficult because continental *R. nigromaculata* and *R. brevipoda* have forms that differ from these. However, both forms of chromosome 1 somewhat resembled those of chromosome 6 in their distribution pattern. Therefore, the A-form is also probably older than the B-form in chromosome 1.

In view of the probable migration course of *R. nigromaculata* and the distributional extent of the B-forms, the B-form of each lampbrush chromosome is presumed to have occurred in the order of chromosomes 11, 1 and 6, 13, and lastly 7. The B-form of chromosome 7 seems to have occurred toward the Würm glacial stage, because it is very similar in distribution pattern to the A-form of chromosome 11. The B-form of chromosome 13 probably occurred before the Würm glacial stage and spread in this stage, judging from the absence in the Eastern Honshu and Chubu groups. This is because the western Honshu, Shikoku, and Kyushu areas united during the Würm glacial stage, since the Inland Sea dried up owing to marine regression [4]. The B-forms of chromosomes 1 and 6 probably spread during the glacial stages before the Würm glacial stage. The B-form of chromosome 11 spread at the beginning of the migration of *R. nigromaculata*.

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